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ORIGINAL PAPER

Culling phenotypically inferior trees in seed production area enhances seed and seedling quality of *Acacia auriculiformis*

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Abstract: Improvement in seed and seedling quality of Acacia auriculiformis after culling phenotypically inferior trees was studied in a 6-year old seed production area (SPA). A 5-ha plantation was identified, of which 2.3 ha was converted into SPA. The initial stocking, 1 612 trees·ha^{-1}, was thinned down to 982 trees·ha^{-1} based on growth characteristics. The following fruiting season, seeds were collected from 10 randomly selected trees in culled and non-culled stands, and seed physical characters, germination and seedling traits were assessed. Seed weight, seed thickness and percentage germination increased by 32.1%, 4.43% and 22.37%, respectively in the culled stand compared to the non-culled stand. Culling also increased the speed of germination, seedling dry weight and seedling vigor index. Heritability values were high for seed weight (0.974) and seed thickness (0.948) while medium values were observed for percentage germination (0.577) and total dry weight (0.534). Predicted genetic gain was 11.13% and 11.22% for seed weight and percentage germination, respectively. The actual gain was 32.1, 51.9 and 22.9% for seed weight, percentage germination and total dry matter, respectively. In conclusion, SPAs established by culling inferior trees could serve as sources of good quality seeds for reforestation programs until genetically improved seeds are made available.

Keywords: genetic gain; germination; seed weight; image analyzer; tree improvement

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Introduction

The success of any tree planting program, among others, hinges on continuous supply of high quality seeds for growing the desired stock in nurseries or for direct sowing in the field. Seed quality is a multiple concept encompassing the physical, physiological and genetic attributes that can determine the performance of the seed when sown or stored (Hampton 2002). In forest tree seed, the genetic quality is particularly important because any anomalies cannot be detected early owing to the long life span of tree growth. Establishment of seed orchards using superior or plus-trees is the most common and cost-effective way of ensuring sustainable supply of genetically improved seeds (Zobel and Talbert 1984; Varghese et al. 2000). Genetically improved seeds are produced from focused tree improvement programs in few important pulpwood and multipurpose species (Burley 1980; National Research Council 1991; Simons 1992; Kanowski 1993; Barnes and Simons 1994).

Tree improvement programs, however, require substantial time for delivery of output because of the long rotation period of trees. Thus, seed production areas (SPAs) are interim approaches and can be established easily for producing quality seeds in short period until genetically improved seeds are produced through genetic improvement programs. Good seeds are obtained from appropriately sited and well-managed SPAs of many tropical acacias and eucalypt species within 4–5 years of planting (Harwood et al 1996). This is particularly important where resources for tree breeding are limited or where a major commitment cannot be justified (Namkoong et al. 1980). In few low priority species, when seeds are required in very large quantity, the seed demand can be entirely met from SPAs (Nienstaedt and Kang 1983).

SPAs are established by culling inferior trees and allowing phenotypically superior trees for cross pollination. The expected genetic gain from these seeds is small when compared to seeds produced from genetic improvement programs (Mandal et al. 1998). The realizable gain from thinning was reported to be

2%–3%, which is half of the theoretical gain that can be attained in closely spaced orchards (Rosvall et al. 2001). Relatively higher gain can be realized after culling of inferior trees in SPAs for stem form than growth characters (Gansel 1967; Kjaer and Sam 1996). Although the genetic gain obtained is low, they are reliable sources of well-adapted seeds produced at reasonable cost (Zobel and Talbert 1984; Shelbourne 1992).

Acacia auriculiformis A. Cunn. ex Benth is a potential timber and pulp wood species native to Australia but widely planted throughout South East Asia. Tree improvement programs for this species were started in 1984, and several international provenance and progeny trials were established over a wide range of environments and growth superiority of different provenances were reported (Pinyopusarerk 1987; Harwood et al. 1991; Coles et al. 1994; Puangchit et al. 1996). The species is pollinated mainly by Trigona, Apis and members of Colletidae (Sedgley et al. 1992). The flowers are small and arranged in elongated spicate inflorescence. The pollen grains are polyads consisted of 16 grains. It is mainly an outcrossing species (Moran et al. 1989) although some degree of self-compatibility was reported after isozyme analysis (Wickneswari et al. 1989). There are many SPAs established in India and elsewhere but only few studies on the actual gain obtained through these SPAs are reported. An understanding of the magnitude of gain achieved from SPAs is essential before embarking further tree improvement efforts. This study reports the improvement in seed and seedling quality of *Acacia auriculiformis* in a SPA established in Chettikulam village at Chalakudi in Kerala state in India.

Materials and methods

Seed production area

A 6-year-old *Acacia auriculiformis* plantation established using seed source from Australia was used for the study. The total area of the plantation was 5 ha with initial density of 1 612 trees·ha^{-1}. The trees were assessed for total height, diameter at breast height, stem straightness and general health. Height and diameter values were converted into scores based on superiority percentage with a maximum score of 15 and 20, respectively. Stem straightness and general health of the trees were scored for a maximum value of 10 and 5, respectively. The trees were ranked based on the total score and trees below a score of 25 were culled. The final density after culling was 982 trees·ha^{-1}. A part of the plantation (2.7 ha) was preserved to compare the improvement in the quality of seeds and seedlings produced from the culled stand (2.3 ha).

Seed collection and assessment of seed characters

In each of the culled and non-culled stands, 10 trees were randomly marked for seed collection. Trees around and between the plots were not selected for 10 rows to reduce the pollen contamination. Seeds were collected from the identified trees in culled and non-culled stands and used in the study. Samples of 500 seeds from culled and non-culled stands each were taken randomly. Seed physical characters, 2D surface area (cm^2), length (cm), breadth (cm), perimeter (cm), roundness and fullness ratio, were assessed using Image analyzer (Leica Quantimet called QWin 500). Seeds were spread in a platform and images were taken into the software called QWin using a CCD camera. The images were then calibrated to actual scale. The calibrated images were measured for the above-mentioned traits using QWin. The 2D surface area was calculated as the area of a seed occupied in the calibrated 2D image. Length and breadth of the seeds were directly measured in QWin, and aspect ratio was computed as the ratio of length to breadth. Roundness is a shape factor, which gives minimum value of unity for a circle. It was calculated as follows:

$$Roundness = \left(\frac{(Perimeter)^2}{(4 \times 2D\,surface\ area \times 1.064)} \right)$$

An adjustment factor of 1.064 was used to correct the perimeter for the effect of the corners produced by the digitization of the image. Fullness ratio, also a shape factor, was computed using the formula given below.

$$Fullness\ ratio = \sqrt{\frac{2D\ surface\ area}{convex\ area}}$$

In addition, seed weight was determined taking 100 seeds in eight replications and expressed as 1000-seed weight (International Seed Testing Association, 2003). Seed thickness was measured for 500 seeds using vernier caliper.

Germination test

A sample of eight replications each with 100 seeds was randomly taken for germination test. Prior to sowing, seeds were treated with hot water and soaked over night to overcome dormancy. Thereafter seeds were sown in sterilized sand trays. The trays were watered every morning. Seedling emergence was counted everyday for 30 days. The following parameters were determined: Percentage germination, germination energy (GE) and germination value. Percentage germination is the proportion of total number of germinated seeds to that of sown seeds, expressed in percentage. GE, also expressed in percentage, is computed as the proportion of germinated seeds after 17 days to that of total germinated seeds after 30 days. GE is one of the commonly employed indices of speed of germination (Robertson 1971). Germination value was calculated following the method given by Djavanshir and Pourbeik (1976).

Measurement of seedling characters

After completion of the germination test, 100 seedlings each were taken out from culled and non-culled groups. These seedlings were studied for various seedling characters: shoot dry

weight, root dry weight, total dry matter and vigor index. The vigor index was calculated as given below (Abdul-Baki and Anderson 1973).

Vigor index = Germination % × Total dry matter per seedling in grams

Data analyses

To get an over view of the improvements in growth characteristics and general health of standing individuals in culled and non-culled stands, a complete inventory was carried out to assess height and diameter growth, and selection differentials and percentage improvement were computed. Seed and seedling characters of the culled and non-culled stands were tested for significant difference using t-test. Coefficient of variation as percentage of mean for seed and seedling characters was estimated separately for culled and non-culled area. Broad-sense heritability and genetic gain values for 1000-seed weight, seed thickness, percentage germination and total dry weight of the seedlings (traits that showed significant differences) were estimated based on half-sib family of the non-culled stand, and percentage improvement due to culling for these characters were quantified. Broad-sense heritability (H^2) and predicted genetic gain values were estimated as follows:

$$H^2 = \sigma^2 g / \sigma^2 p \tag{1}$$

Genetic gain = $i\sigma_p H^2$

where, $\sigma^2 g$ is the genotypic variance, $\sigma^2 p$ the phenotypic variance, i the selection intensity, and σ_p is the phenotypic standard deviation. The selection intensity was estimated based on culling intensity (39%). The selected proportion was 0.61, thus the selection intensity was calculated as 0.64 (Referring to the table given by Zobel and Talbert 1984). The observed improvement percentage (I) due to culling was also computed for 1000-seed weight, seed thickness, percentage germination, and total seedling dry weight as follows:

$$I = (\mu_s - \mu_0) \times 100 \tag{2}$$

where, μ_s is the average for traits of the selected population (culled stand), and μ_o is the average for traits of the base population (non-culled stand).

Results

Assessment of standing trees in culled and non-culled stands showed that culling phenotypically inferior trees in a seed production area resulted in remarkable improvement in diameter and height followed by straightness and general health conditions (Table 1). Complete enumeration of trees in non-culled and culled areas showed improvement in selection differential of 1.52 m and 5.8 cm in height and diameter, respectively.

Table 1. Height, diameter, straightness and general health of trees in the non-culled and culled stands and estimated improvements after culling.

Treatment	Height (m)	Diameter (cm)	Straightness (score)	Health (score)
Non-culled stand	8.06	26.90	6.70	3.80
Culled stand	9.58	32.70	7.20	4.10
Selection differential	1.52	5.80	0.50	0.30
% improvement	18.86	21.56	7.46	7.89

Among the different seed physical characteristics examined, 1000-seed weight and seed thickness were significantly higher in seeds collected from the culled stand than the non-culled stand (Table 2). About 32.1% and 4.43% increase in seed weight and seed thickness, respectively was observed in the culled stand. All germination parameters assessed displayed significant differences between culled and non-culled stands (Table 3). The percentage germination increased from 43.13% in the non-culled stand to 65.50% in the culled stand. The speed of germination of seeds collected from culled stand was significantly higher than seeds collected from non-culled stands, as shown by the higher germination energy and germination value (Table 3). For all seed physical characters, the coefficient of variation was low for culled stand compared to the non-culled stand. On the contrary, the coefficients of variation for germination parameters were higher for culled than non-culled stands.

Table 2. Physical characteristics of *Acacia auriculiformis* seeds collected from non-culled and culled stands

Stands	2D surface area (cm^2)	Seed length (cm)	Seed breadth (cm)	Seed thickness (mm)	Roundness	Aspect ratio	Fullness ratio	1000-seed weight (g)
Culled	0.12 (15.5)	0.458 (8.42)	0.343 (9.68)	1.746 (9.15)	1.134 (3.64)	1.343 (8.92)	0.971 (1.08)	20.66 (2.99)
non-culled	0.118 (18.3)	0.454 (9.55)	0.341 (11.79)	1.672 (10.93)	1.132 (4.01)	1.340 (9.13)	0.972 (1.16)	15.64 (5.51)
t_{cal}	1.733	1.673	0.876	4.812*	0.921	0.366	0.872	13.372*

* Significant; $t_{[0.05, 998)} = 1.96$ for all characters except 1000-seed weight ($t_{[0.05, 14)} = 2.145$); Values in parenthesis are CV (%).

Seedling characters exhibited significant differences in response to culling of phenotypically inferior trees in the seed production area. Among the characters, significant improvement was observed in shoot dry weight, total dry matter, and seedling vigor index (Table 4). Seedling vigor index and total dry matter were nearly doubled for seedlings produced from seeds collected in culled stands compared with those raised from seeds obtained from non-culled stands. The variability in seedling characters, as

shown by CV, was higher in seedlings from culled stands than in non-culled stands

Table 3. Germination of *Acacia auriculiformis* seeds collected from non-culled and culled stands.

Stands	Percentage germination	Germination energy	Germination value
Culled	65.5 (8.8)	27.9 (8.9)	8.9 (6.2)
non-culled	43.1 (7.7)	18.6 (5.5)	4.0 (3.5)
t_{cal}	9.497*	8.352*	9.281*

* Significant; $t_{[0.05,\ 14)}$ = 2.145 for all germination parameters; Values in parenthesis are CV (%)

Table 4. Vigor index and dry matter production of *Acacia auriculiformis* seedlings raised from seeds collected from non-culled and culled stands.

Stands	Seedling vigor index	Total dry matter (mg)	Shoot dry matter (mg)	Root dry matter (mg)
Culled	16.51 (10.31)	252 (8.86)	215 (8.15)	37 (15.79)
non-culled	8.84 (4.36)	203 (3.92)	172 (4.92)	33 (10.55)
t_{cal}	8.179*	5.713*	6.335*	1.828

* Significant, $t_{[0.05,\ 14)}$ = 2.145 for all seedling parameters; Values in parenthesis are CV (%).

Heritability estimate shows that 97.4% and 95% of the total phenotypic variations in 1000-seed weight and seed thickness respectively were attributed to genetic variation (Table 5). Medium heritability values were observed for percentage germination (0.577) and total dry weight (0.534). Relatively high genetic gain was predicted for 1000-seed weight and percentage germination followed by seed thickness and total dry weight (Table 5). Substantial gain was realized for 1000-seed weight, percentage germination and total dry matter after culling against the predicted gain (Table 5). For seed thickness, realized gain was lower (4.4%) than the predicted gain (6.49%).

Table 5. Heritability and predicted genetic gain (%) for selected seed and seedling parameters and realized improvement (%) due to culling

Seed/seedling Characters	Broad sense Heritability	Predicted Genetic gain	Realized Improvement
1000-Seed weight	0.974	11.13	32.1
Seed thickness	0.948	6.49	4.4
Percentage germination	0.577	11.22	51.9
Total dry matter	0.534	6.06	22.9

Discussion

Culling 39% of trees based on height, diameter, straightness and general health has shown improvement in growth performance of the culled stand (Table 1). Among these traits, improvement was high in diameter after culling. A similar higher improvement in diameter (28%) than height (16%) after culling was reported for *Pinus taeda* (Paludzyszyn et al. 2002). In the present study, high intensity of selection (61%) has resulted in very low selection differential of 1.52 m for height and 5.8 cm for diameter. The culling intensity in SPA is decided considering the requirement of sufficient crown space for better flowering as well as retaining adequate number of trees for optimal cross pollination. In an earlier study made on Eucalyptus hybrid, culling by selecting 8.33% of trees realized a high selection differential of 12.63 m for height and 33.91 cm for girth at breast height (Tewari et al. 2001).

Seed weight and seed thickness were significantly higher in the culled stand than the non-culled stand (Table 2). The increase in seed weight and thickness in the culled stand might be attributed to increased spacing, cross pollination and improved growing conditions in the plantation due to removal of inferior trees. Increased spacing was reported to improve seed yield and 1000-seed weight in *Albizia lebbeck* (Matias 1998). Thinning showed improvement in flowering and seed production in *Eucalyptus dunnii* (Higa et al. 2001) and seed size and weight in *Alnus cordata* (Giovannini et al. 1990). There are also several studies which show increase in seed weight due to cross pollination (Pirags 1976; Magini et al. 1988; Venkatesh and Thapliyal 1993; Vaughton and Ramsey 1997). In addition, a reduction in CV for physical characters of seeds collected from culled stand was observed, suggesting that removal of inferior trees resulted in uniform population in the culled stand, which in turn resulted in uniformity of seed characters.

Culling of phenotypically inferior trees resulted in higher seed germination (Table 3). This might be related to increased allocation of resources for seed production as a result of increased spacing among seed trees retained in the culled stand. CV for percentage germination was found to increase in the culled area possibly because cross pollination is favored among the retained individuals. *A. auriculiformis* is an insect pollinated species. Primarily, insect pollinated species favors self-pollination because during anthesis, insects crawl from anther to stigma of same flower and the tendency of the insects to forage mostly among flowers or inflorescences of the same tree resulted in lack of heterozygosity in teak (Tangmitcharoen and Owens 1996). At the same time, reducing the number of seed trees increases the probability of out-crossing (Mencuccini et al. 1995). In insect pollinated *Phlox drummondii*, a decrease in plant density was observed to increase the out crossing rate (Watkins and Levin 1990).

Seedlings from the culled stand showed marked improvement in dry matter production and vigor (Table 4). Study conducted on loblolly pine showed 17% more height growth in seedlings of seed production area when compared to other nursery stocks (Easley 1967). In *Pinus elliottii*, the response to selection of trees for straightness was significantly higher while significant difference was not observed for growth characters between seeds collected from SPA and adjacent area (Gansel 1967). In the present study, CV for seedling characters has increased in the culled area, indicating that cross-pollination is improved due to random movement of insect pollinators after removal of inferior trees and

increased spacing.

Relatively high heritability estimates were found for 1000-seed weight and seed thickness (Table 5). Similarly the predicted genetic gain and the realized improvement were high for seed weight. Nyoka and Tongoona (2001) suggested that cone and seed yields are under moderate to strong genetic control and that family selection can bring substantially improvement. Similar high heritability and genetic gain values for seed weight have been reported for *Sapindus trifoliatus* (Kumar and Devar 2002). Percentage germination and total dry weight of seedlings showed moderate heritability estimate. Individual tree heritability estimates for growth traits are typically low to moderate (Cotterill and Dean 1990). However, high genetic gain was predicted for percentage germination (11.22%). Culling experiment conducted in *Acacia mearnsii* resulted in 2.4% genetic gain in DBH and 3.1% in tannin from selection of the best 30% of families for both traits (Resende et al. 1991). Culling through retrospective selection index for bole length and stem straightness in *Acacia mangium* resulted in 4 - 6% gain for stem form and less than 3% for growth traits (Kurinobu et al. 1996). In *Cunninghamia lanceolata*, genetic gains achieved for height, diameter and volume after 3 roguing treatments were 18.24%, 18.43% and 30%, respectively (Chen et al. 1985).

The actual improvement realized by culling 39% of inferior trees was three times high for seed weight, five times for germination and four times for total dry weight. This is because the genetic gain was predicted for the growing conditions of the non-culled area. Culling of inferior trees results in more space, sunlight, moisture and root growth (Sword et al. 2000) for the remaining trees. The improved growing condition of the culled area coupled with genetic thinning resulted in improvement of seed weight over the predicted genetic gain. Similar study conducted in *Alnus cordata* showed improvement in seed weight after culling (Giovannini et al. 1990). The improvement in seed weight would have resulted in higher germination and total dry weight of seedlings. The influence of seed weight on germination and seedling biomass are well documented (Manga and Sen 1996; Mahadevan et al. 1999; Nizam and Hossain 1999; Jiang and Jiang 2000; Khan et al. 2001; Samidha et al. 2002; TeKrony 2003). As a whole, seed production areas established through culling of phenotypically inferior trees can serve as an interim source of genetically improved seed lots. However, care should be taken to avoid excessive thinning which might reduce the genetic diversity in future tree crops.

Conclusions

The study reveals that culling of inferior trees in a seed production area improves seed weight, percentage germination and speed of germination. The quality of seedlings is also improved after culling. Substantial genetic gain is achieved by culling of inferior trees. Therefore, seed production areas established by culling inferior trees could serve as sources of good quality seeds for immediate reforestation programs until genetically improved seeds are made available through improvement program.

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